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Abstract

Zinc(II) ions are redox-inert in biology. Yet, their interaction with sulfur of cysteine in cellular proteins can confer ligand-centered redox activity on zinc coordination sites, control protein functions, and generate signalling zinc ions as potent effectors of many cellular processes. The specificity and relative high affinity of binding sites for zinc allow regulation in redox biology, free radical biology, and the biology of reactive species. Understanding the role of zinc in these areas of biology requires an understanding of how cellular Zn2+ is homeostatically controlled and can serve as a regulatory ion in addition to Ca2+, albeit at much lower concentrations. A rather complex system of dozens of transporters and metallothioneins buffer the relatively high (hundreds of micromolar) total cellular zinc concentrations in such a way that the available zinc ion concentrations are only picomolar but can fluctuate in signalling. The proteins targeted by Zn2+ transients include enzymes controlling phosphorylation and redox signalling pathways. Networks of regulatory functions of zinc integrate gene expression and metabolic and signalling pathways at several hierarchical levels. They affect enzymatic catalysis, protein structure and protein-protein/biomolecular interactions and add to the already impressive number of catalytic and structural functions of zinc in an estimated three thousand human zinc proteins. The effects of zinc on redox biology have adduced evidence that zinc is an antioxidant. Without further qualifications, this notion is misleading and prevents a true understanding of the roles of zinc in biology. Its antioxidant-like effects are indirect and expressed only in certain conditions because a lack of zinc and too much zinc have pro-oxidant effects. Teasing apart these functions based on quantitative considerations of homeostatic control of cellular zinc is critical because opposite consequences are observed depending on the concentrations of zinc: pro- or anti-apoptotic, pro- or anti-inflammatory and cytoprotective or cytotoxic. The article provides a biochemical basis for the links between redox and zinc biology and discusses why zinc has pleiotropic functions. Perturbation of zinc metabolism is a consequence of conditions of redox stress. Zinc deficiency, either nutritional or conditioned, and cellular zinc overload cause oxidative stress. Thus, there is causation in the relationship between zinc metabolism and the many diseases associated with oxidative stress.

Keywords	zinc; pro-oxidant; pro-antioxidant; zinc signalling; redox signalling; redox zinc switches
Taxonomy	Physiology, Toxicology, Biochemistry
Manuscript category	I have taken advantage of "Your Paper-Your Way" and my manuscript is a PDF file

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FRBM_2018_860

Dear Professor Mann,

I appreciate the expeditious handling of my manuscript and obviously I am delighted about the positive response of the reviewers.

Submitted is a revised manuscript that incorporates all the reviewers' comments. Additional typographical errors have been addressed.

A new figure (Fig. 8) that summarizes the effects of zinc ions on cell signalling has been prepared and all figures have been enlarged (inserted as TIF files in the text).

R 1

The reference suggested by the reviewer has been added [177].

R 2

(1) Term "periodic table" now used
(2) Typos corrected. BUT: It is quaternary structure. I know it is irritating for someone trained in the origins of word – the "r" got somehow lost in the evolution of the English language.
(3) Correction made, i.e. that these considerations are in the context of the cellular milieu
(4) Inhibition constant specified

R3

1-4 The word "scavenge" has been replaced with "react with" and the last sentence of p. 12 has been deleted.

Yours sincerely,

Wolfgang Maret

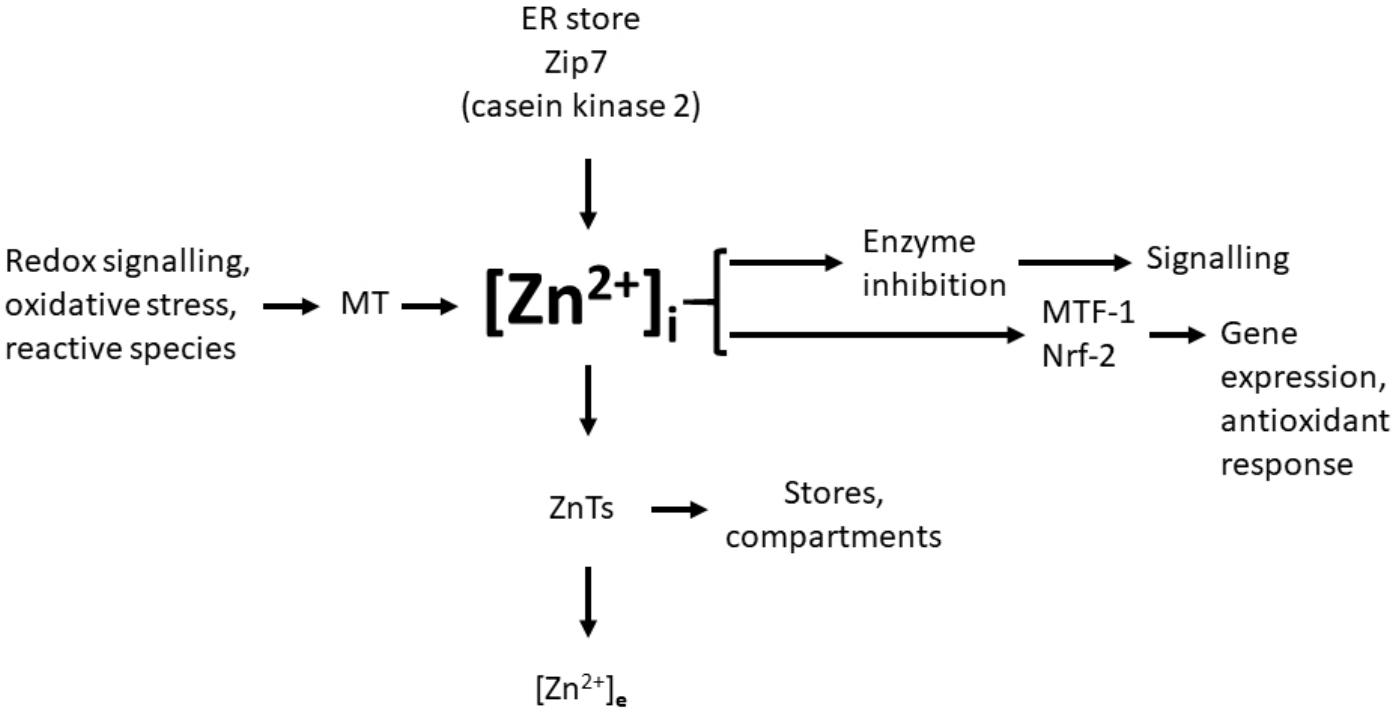
Highlights

• Redox-inert zinc ions regulate some aspects of redox biology and the biology or reactive species.

• Control of cellular zinc homeostasis determines antioxidant-like or pro-oxidant effects.

• Zinc deficiency and overload cause oxidative stress.

• Disorders of zinc metabolism are a cause or consequence of diseases associated with oxidative stress.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The redox biology of redox-inert zinc ions

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Keywords: zinc; pro-oxidant; pro-antioxidant; zinc signalling; redox signalling; redox zinc switches

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Abbreviations

ER, endoplasmic reticulum; GCL, glutamate cysteine ligase; MT, metallothionein; MTF-1, metal regulatory element (MRE)-binding transcription factor-1; NOS, nitric oxide synthase; PARP, poly (ADP-ribose) polymerase; PKC, protein kinase C; ZnT, zinc transporter

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Abstract. Zinc(II) ions are redox-inert in biology. Yet, their interaction with sulfur of cysteine in cellular proteins can confer ligand-centered redox activity on zinc coordination sites, control protein functions, and generate signalling zinc ions as potent effectors of many cellular processes. The specificity and relative high affinity of binding sites for zinc allow regulation in redox biology, free radical biology, and the biology of reactive species. Understanding the role of zinc in these areas of biology requires an understanding of how cellular Zn^{2+} is homeostatically controlled and can serve as a regulatory ion in addition to Ca^{2+}, albeit at much lower concentrations. A rather complex system of dozens of transporters and metallothioneins buffer the relatively high (hundreds of micromolar) total cellular zinc concentrations in such a way that the available zinc ion concentrations are only picomolar but can fluctuate in signalling. The proteins targeted by Zn^{2+} transients include enzymes controlling phosphorylation and redox signalling pathways. Networks of regulatory functions of zinc integrate gene expression and metabolic and signalling pathways at several hierarchical levels. They affect enzymatic catalysis, protein structure and protein-protein/biomolecular interactions and add to the already impressive number of catalytic and structural functions of zinc in an estimated three thousand human zinc proteins. The effects of zinc on redox biology have adduced evidence that zinc is an antioxidant. Without further qualifications, this notion is misleading and prevents a true understanding of the roles of zinc in biology. Its antioxidant-like effects are indirect and expressed only in certain conditions because a lack of zinc and too much zinc have pro-oxidant effects. Teasing apart these functions based on quantitative considerations of homeostatic control of cellular zinc is critical because opposite consequences are observed depending on the concentrations of zinc: pro- or anti-apoptotic, pro- or anti-inflammatory and cytoprotective or cytotoxic. The article provides a biochemical basis for the links between redox and zinc biology and discusses why zinc has pleiotropic functions. Perturbation of zinc metabolism is a consequence of conditions of redox stress. Zinc deficiency, either nutritional or conditioned, and cellular zinc overload cause oxidative stress. Thus, there is causation in the relationship between zinc metabolism and the many diseases associated with oxidative stress.

About thirty years ago a review article in this journal discussed a physiological role of the essential micronutrient zinc as an antioxidant [1]. Largely from a nutritional perspective, it summarized how zinc protects against chemical and physical insults. Two molecular mechanisms were thought to underlie such a function: i) zinc binds to sulfhydryl groups and protects them from modification by oxidants and reactive species, and ii) zinc competes with iron and copper in their coordination environments and suppresses their redox activity that can generate damaging hydroxyl radicals ($OH^{\cdot}$) through Fenton chemistry. The authors state that *physiological* concentrations of zinc do not seem to have antioxidant effects while *supraphysiological* concentrations have antioxidant-like effects in organelle- and cell-based systems and *pharmacological* doses protect the liver and the lung against the deleterious effects of pro-oxidants. They discuss that zinc deficiency causes damage to cellular membranes, suggest that oxidative stress† is a component, and conclude that zinc has a general, stabilizing and inhibitory effect on many cellular systems and that critical antioxidant functions of zinc may still be uncovered. Another major review ten years later included the now known damaging effects of zinc deficiency to DNA and proteins in addition to cellular membranes, and added from a medical perspective that zinc can reduce postischemic injury to a variety of tissues [2]. It concluded that “although the evidence for the antioxidant properties of zinc is compelling, the mechanisms are still unclear.” Key questions regarding the actions of zinc in redox biology remained

from these articles, namely: What is the molecular basis for the effects of zinc on redox biology? Are the observed effects general or specific? And at which concentrations does zinc have antioxidant-like effects? In the years following these reviews, it became clear that the entire biological dose-response relationship must be considered to interpret the effects of zinc on redox biology because pro-oxidant effects are observed when the concentrations of zinc are either too low or too high. Also, the discovery of molecular mechanisms demonstrated that the above postulated mechanisms are not the major ones accounting for the antioxidant-like effects and that zinc has a more general role as a regulatory (signalling) ion in controlling some aspects of redox biology.

In this article, the developments in the last two decades are summarized and discussed. Significant findings in zinc biology have strengthened the link between zinc and redox biology and provide a quite different interpretation of the earlier literature. It was discovered how tightly cellular zinc is regulated and how this regulation is used for biological control. The discoveries extended the protective effects of zinc to many organ systems, making perturbations of zinc metabolism inevitably an important consideration in the pathogenesis of all the diseases linked to redox stress or changes of cellular redox state. The scientific literature on the topic is vast. Therefore, it is an unattainable goal to even summarize just the many reviews. This review therefore takes a different approach. It provides a framework based on our present knowledge of zinc biology. Such knowledge will allow interpretation of the plethora of reported phenomena. From understanding the remarkable roles of zinc in biology, an understanding will develop why Nature has chosen the redox-inert zinc ion to control aspects of redox biology. Only then the article will briefly allude to the huge implications for the complex, system-level functions of zinc in the redox biology of health and disease.

1. Redox-inert zinc (Zn^{2+})

In biology, zinc always remains in the Zn(II) valence state and can neither oxidize nor reduce another substance. Therefore, the statement that zinc is redox-inert in biology should be a conversation stopper for a journal dedicated to redox biology. Yet, somewhat paradoxically, it turns out that zinc ions (Zn^{2+}) have a central role in the control of redox biology.

Critically important is an assessment of what is meant by considering redox-inert zinc an antioxidant, a connotation adopted by many scientists and the public to describe the characteristics of zinc. A major textbook states that “antioxidant is a term widely used but surprisingly difficult to define clearly” and then provides a definition as “any substance that delays, prevents, or removes oxidative damage to a molecule” [3]. In the case of zinc, the addition or removal of zinc to/from a biological system overcomes a pro-oxidant state caused by a lack of zinc or an excess of zinc. When employed for zinc, the term “antioxidant” is misleading because the redox effects can only be indirect, and the effects depend on zinc concentrations. Therefore, in analogy to the term pro-oxidant, the term pro-antioxidant has been suggested [4]. These dual functions of zinc in redox metabolism and the need to consider its concentrations throughout the entire dose-response curve have been captured in a title of a review article that described zinc as an “essential toxin” [5]. The description incorporates the fact that zinc is an essential micronutrient and yet can become a toxin when zinc ions are released intracellularly from proteins and their concentrations are too high without necessarily changing total zinc concentrations. Furthermore, one needs to distinguish whether any redox effects are due to the zinc already present, any zinc added or any perturbation of intracellular

equilibria and re-distribution of zinc. In this article, I will consider the full dose-response curve that covers nutrition, pharmacology, and toxicology (Figure 1).

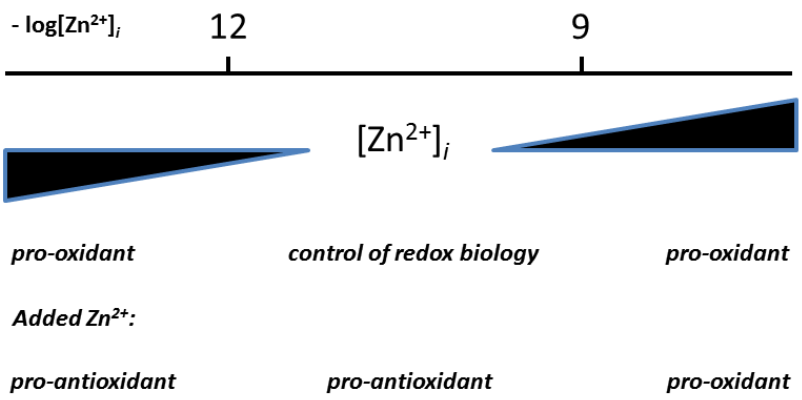

Figure 1 The redox effects of redox-inert zinc ions. Zinc present in and added to a biological system can have pro-antioxidant or pro-oxidant effects. Critically important are the control of the very low cellular concentrations of "free" zinc ions, $[Zn^{2+}]_i$ and their functions as signalling ions. The concentration is given on a logarithmic scale and corresponds to picomolar concentrations in the physiological range in contrast to the total cellular zinc concentration, which is in the range of hundreds of micromolar.

In defining the functions of zinc in redox biology, we need to link the concentrations of both zinc and reactive species to either physiology or pathophysiology. The vast literature on the effects of zinc on redox biology must to be read, understood, and interpreted based on what is now known about zinc in biology. With its redox-inert characteristics, zinc is functionally closer to redox-inert magnesium and calcium than to the nutritionally essential redox-active transition metal ions manganese, iron, and copper, and thus has a special position in the periodic table when related to biology (Figure 2). Among the three redox-inert divalent metal ions (Mg^{2+}, Ca^{2+}, Zn^{2+}) it is the one with the strongest interaction with donor atoms of ligands. The consequence of such strong binding (affinity) is that zinc is a potent effector at correspondingly lower concentrations. Accordingly, the three metal ions can cover a wide range of concentrations from millimolar to picomolar in biological regulation.

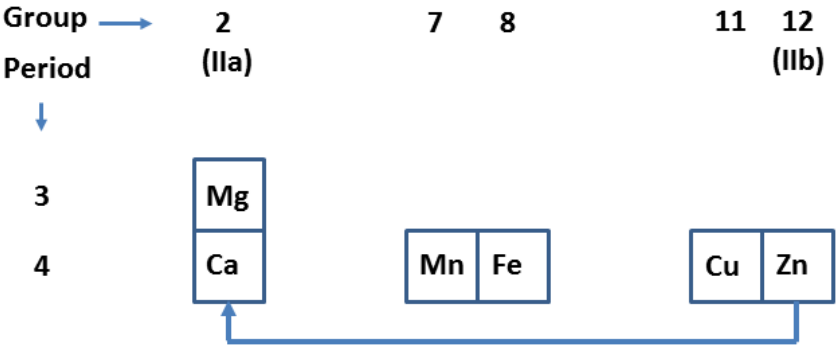

Figure 2 Position of zinc in the periodic table. While zinc is part of the first transition metal series/period 4 - though not a transition metal ion itself - its redox-inertness in biology puts it functionally closer to magnesium and calcium. The chemical relationship is also expressed by referring to group 2 as group IIa and group 12 as group IIb. Zinc has higher affinity to biological sites than magnesium and calcium. Accordingly, its available ("free") concentrations are lower.

2. Zinc biology

The functions of zinc bear on virtually any biological process. They relate to proteins and are catalytic, structural, and regulatory. The field developed in this order of functions, zinc enzymes, zinc proteins, and zinc ions as modulators of enzymes and proteins [6]. The first two are relatively well defined in many enzymes and as a way of organizing the structure of proteins. What is understood by "regulatory" has not become evident until relatively recently, however. Regarding enzymatic catalysis, zinc was discovered in carbonic anhydrase about eighty years ago, twenty years before it was recognized to be an essential micronutrient for humans [7,8]. The discovery in many more enzymes followed so that hundreds of zinc enzymes in all six classes became known [9]. Discovery was mostly by time-consuming isolation of proteins from their natural sources and characterization of their metal content before the advent of molecular cloning and heterologous protein expression. A remarkable development from about the 1980s onward was the discovery of zinc in transcription factors, the so-called zinc finger proteins and related motifs. In these proteins, zinc has primarily a structural role [10,11]. Not biochemical preparations but computational approaches (bioinformatics) began to make critical contributions. The reason for the success of bioinformatics is that the presence of a putative structural zinc site is more readily recognized than a catalytic site in the sequence of proteins because structural sites usually have a characteristic signature with four rather closely spaced amino acids, the side chains of which serve as ligands to zinc. Protein and nucleic acid sequence databases were screened for such signatures and many more proteins were predicted to be zinc metalloproteins [12]. For both catalytic and structural sites, with few exceptions, the ligands are provided by the side chains of Asp/Glu, His, and Cys. Among those amino acids, Cys is the one most frequently employed as a ligand, but not in catalytic sites [13]. Signatures of zinc-binding amino acids and "spacers" between them allow searching sequence databases for possible zinc-binding sites. In this way, about a thousand proteins containing zinc fingers or related motifs were identified [14]. It demonstrated that zinc sites are a major principle of protein structure and biomolecular interactions, namely forming domains of proteins that can interact with DNA, RNA, other proteins, and lipids. Furthermore, many examples emerged where zinc is not part of the structure in the protein but binds between subunits to control the assembly of proteins in quaternary (same protein) or quinary (different proteins) structure. Cysteines also feature as ligands in such sites.

Once the sequences of entire genomes became available, they could be mined for possible zinc-binding sites. Investigators arrived at the remarkable estimate that over three thousand proteins in humans have functional zinc-binding sites, meaning that every 10th protein is a zinc protein [15]. This data mining approach began a new era with a functional annotation of zinc proteins. The many anecdotal observations in zinc biology turned out not to be a matter of lack of specificity but rather the consequence of the pleiotropic functions of zinc in such a great number of proteins. All of these functions require tight control. Three salient developments provide further information of this

control, made a great impact on the field, and are critically important for discussing the relationship between zinc and redox biology.

2.1 Proteins regulating zinc. Control of cellular homeostasis

To ascertain that such a large number of zinc proteins receive their zinc at the correct time and space in the cell, dozens of proteins, whose sole function is to handle metal ions in the cell and subcellularly, are required. In humans, minimally twenty-four transporters of two protein families control the passage of zinc through cellular membranes: 14 ZIP (Zrt- and Irt-like proteins) proteins, which are zinc importers and mostly located on the plasma membrane, and 10 ZnT (zinc transporter) proteins, which are zinc exporters and mostly located on intracellular membranes [16]. One subgroup of ZnTs transports zinc into vesicles for storage or exocytosis of the vesicular content together with zinc. The best characterized systems are synaptic vesicles in some neurons, insulin granules of pancreatic β-cells, and granules in epithelial cells of the mammary gland. In addition, about a dozen metallothioneins (MTs) bind zinc in the cell. This relatively large number of proteins and their control by many pathways constitute a rather complex system that interacts with many aspects of cellular biology.

2.2 Affinities of proteins for zinc and cellular zinc buffering

Quantitative aspects of cellular zinc biology are perhaps least widely acknowledged. They are critical for interpreting the scientific literature on zinc's cellular functions. The total cellular zinc concentration is rather high, in the hundreds of micromolar, and even higher (millimolar) in some subcellular vesicles. Thus, as far as cellular biochemistry is concerned, zinc is not a trace element. The idea of a "trace" comes from the fact that only rather small amounts (2-3 mg) of zinc are needed daily in the diet to maintain a total amount of 2-3 g in a 70 kg human. The bulk of zinc is bound to proteins. The binding capacity and affinity determine how much protein-bound zinc is in equilibrium with zinc that is not bound to proteins. The formalism to describe this binding is rather simple. In analogy to hydrogen ion (proton) buffering (equation 1), there is the concept of metal ion buffering, in this case zinc ion buffering (equation 2).

(1) $pH = pK_a + \log[base]/[acid] = -\log[H^+]$ (Henderson-Hasselbalch equation)

(2) $pZn = pK_d + \log[ligand]/[Zn\text{-}ligand\ complex] = -\log[Zn^{2+}]$

(3) $E = E^o + 0.059\ V/z \log[oxidant]/[reductant]$ (Nernst equation)

The affinities (K_d) of cytosolic zinc proteins for zinc are rather high. Compared to other nutritionally essential metal ions, zinc is a very competitive ion - exceeded only by copper. The consequence of zinc binding to proteins with relatively high affinity is a potency of its biological actions. The high affinity and the relatively large number of binding sites buffer zinc in such a way that the "free" (not protein-bound) zinc ion concentration is only in the picomolar range. It was estimated that in order of the magnesium enzyme phosphoglucomutase not to be zinc inhibited, the "free" zinc ion concentration in muscle must be <32 pM [17]. In erythrocytes, a concentrations of 24 pM was measured [18]. With the advent of fluorescent chelating agents for zinc, a few hundred picomolar were determined in several cell lines [19,20]. Thus, zinc ions are buffered at a pZn of about 10. Buffering has two components. First, in analogy to the definition of pH and the Henderson-Hasselbalch equation (equation 1), it is the pZn value, the "free" zinc ion concentration (equation 2).

Second, it is the buffering capacity, which describes how resistant the pZn value is to change. It is determined by the concentrations of zinc-binding ligands and it is in the micromolar range [19]. Biological buffering also has a kinetic component, referred to as muffling, which describes the increase or decrease of zinc ion concentrations effected by the many transporters controlling cellular homeostasis [21].

Zinc buffering keeps "free" zinc ions in a range of concentrations that make zinc-specific functions in zinc metalloproteins possible as it allows separating the functions of zinc from those of other metal ions, which are buffered in different ranges. Knowledge of this zinc buffering is necessary to understand the interactions of zinc with proteins *ex vivo* and *in vivo*, and to determine the appropriate amounts of zinc for experiments in cell and tissue culture. At least three regions describe the actions of zinc: concentrations that provide apoenzymes with zinc, regulate proteins, and affect proteins that are normally not targeted (Figure 3).

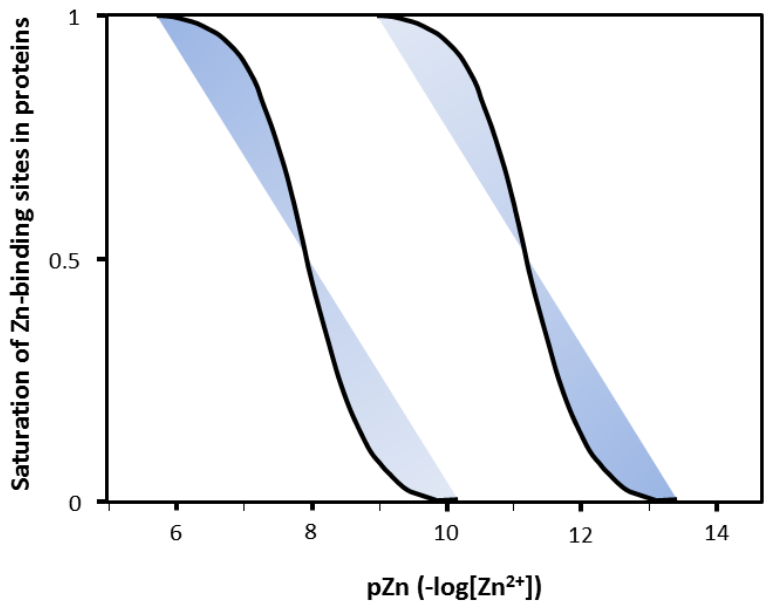

Figure 3 The buffering of cellular zinc. Three regions can be identified (from right to left): a range where not enough zinc ions are available either to provide proteins with zinc or to support regulatory functions of zinc, an optimal range where such functions are supported, and a range where the zinc ion concentrations are too high and interfere with proteins that normally do not depend on zinc.

In addition to the pH value, the redox potential affects the pZn value (equation 3). The ligand coordination environments of zinc provide a functional context as they determine how pH and redox effects are expressed (Figure 4). The pH value affects the protonation of ligands of zinc, increasing or decreasing "free" zinc ion concentrations at more acidic or more alkaline pH, respectively. The redox potential as described by the Nernst equation (equation 3) affects thiols that bind zinc. Thus any redox changes affecting thiols increases or decreases the "free" zinc ion concentrations. In the cellular context, His coordination environments are mainly pH-sensitive, Cys coordination environments are mainly redox-sensitive, and His/Cys environments are both pH and redox-

sensitive, while Asp/Glu coordination environments are independent of either pH or redox conditions.

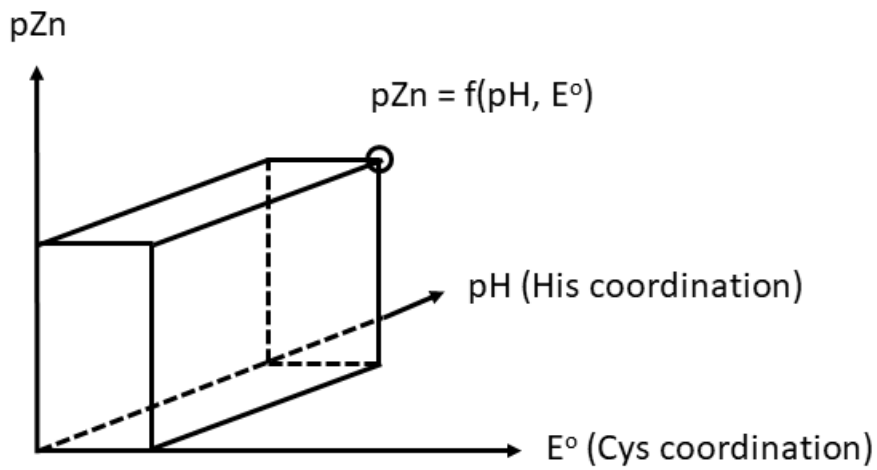

Figure 4 Functions of the ligand donor atoms in the biological coordination environments of zinc and consequences for zinc buffering (pZn). Coordination environments with His are pH-sensitive around physiological pH. Coordination environments with Cys are primarily redox-sensitive. Coordination environments with only Asp/Glu do not show these dependences in the cellular context. Typical pK_a values are 4.4 for Asp/Glu, 6.5 for His, and 8.5 for Cys.

2.3 Zinc regulating proteins. Zinc signalling

In recent years, induced fluctuations of ”free” zinc ion concentrations have been shown to be used in biological regulation. In analogy to calcium signalling, they are referred to as zinc signalling. The chemical nature of “free” zinc ions is not known [22]. Given the strong interaction with ligands, zinc ions most certainly have a ligand environment other than water molecules. In addition to controlling steady-state zinc concentrations, induced fluctuations of zinc ions need to be controlled. A regulatory role of zinc ions was originally established for the zinc-sensing transcription factor MTF-1 (metal regulatory element (MRE)-binding transcription factor-1), which interacts with its six zinc fingers with DNA and controls the transcription of genes when zinc ion concentrations are elevated. The transcriptional response was originally seen mainly as a way of restoring steady-state zinc concentrations by inducing MT (zinc-binding) and ZnT1 (zinc export). However, MTF-1 induces many other genes involved in the response to stress such as hypoxia and oxidative stress [23]. In the last decade, it became clear that zinc has additional regulatory functions beyond gene expression [24,25]. The full impact of zinc ions in regulation is still emerging. There at least three pathways for generating zinc ion signals [26,27]. Zinc ions are exocytosed for autocrine, paracrine and possibly endocrine functions. Zinc ions increase intracellularly when reactive species target zinc thiolate coordination environments, which serve as transducers of the redox signal, or when zinc transporter channels are opened by phosphorylation. Zinc ion fluxes intersect with many signalling pathways, including phosphorylation, calcium, and redox signalling, with huge implications for signal transduction and cell biology:

Stimulus → $[Zn^{2+}]_i$ → effect on targets in phosphorylation, calcium, and redox signalling

Like calcium transients, zinc transients have amplitudes of about one order of magnitude in concentration above baseline values and occur in a zinc-buffered environment. Also like calcium, a more than 4-fold increase is cytotoxic and causes apoptosis.

Some enzymes have been identified as targets of the signalling zinc ions and structurally characterized. The modulation of their activity indicates genuine biological regulation by zinc ion fluxes. Zinc inhibition of enzymes was known for a long time to occur over a range of picomolar (pM), nanomolar (nM), and micromolar (μM) concentrations. The question is which range is physiologically significant. Over sixty years ago, it was reported that picomolar concentrations of zinc inhibit phosphoglucomutase, a magnesium enzyme. The inhibition constant (IC_{50}) is about 2 pM [28]. On the other end of the concentration range, the scientific literature is replete with reports of zinc inhibiting proteins with micromolar and even millimolar inhibition constants, which some investigators consider high affinity sites and discuss as being physiologically meaningful, even in high impact journals. Such interpretations will need to be re-examined as we now know the range of "free" zinc ion concentrations. With quantitative information about the concentrations at which zinc ion transients occur and the affinities of proteins targeted, it will be possible to distinguish physiological redox and zinc signalling from pathological oxidative stress and associated cytotoxic increases in cellular "free" zinc ion concentrations.

The number of regulatory sites for zinc in proteins is not known. Many transient zinc binding sites have been observed. They must be evaluated on the basis of their physiological significance and added to the already impressive number of about three thousand zinc proteins. For example, it was known that zinc inhibits regulatory enzymes such as caspases and protein tyrosine phosphatases [29,30]. However, the inhibition occurs at much lower concentrations than originally reported [31]. Very specific mechanisms for zinc inhibition were discovered. For protein tyrosine phosphatase 1B (PTP1B), which counteracts the kinase activity of the insulin receptor, the inhibition occurs in the closed conformation of the enzyme. The binding site is thought to include the cysteinyl phosphate intermediate and the catalytic aspartate in the active site [32-34]. Zinc inhibition (IC_{50} = 3-17 nM) provides a modulation of activity that differs from the known redox modulation of PTP1B activity, which occurs in the open conformation of the enzyme and involves the non-phosphorylated catalytic cysteine. Zinc locks the enzyme in the closed conformation and makes the catalytic cysteine inaccessible for oxidation. Therefore, zinc inhibition of PTP1B is one example where zinc may protect an enzyme from oxidative inactivation, albeit not by directly binding to the sulfur of the catalytic cysteine. In the case of receptor protein tyrosine phosphatase β (RPTPβ), zinc inhibition is so strong (K_i =21 pM) that the enzyme is thought to be inhibited tonically and needs to be activated by zinc removal [35]. Other PTPs are zinc-inhibited as well, suggesting a general effect of zinc on phosphorylation signalling [36]. PTEN (phosphatase and tensin homolog), for example, has an IC_{50} of 0.6 nM for zinc [37]. In as much as phosphatases are the major regulators in phosphorylation signalling and are modulated by zinc inhibition, other hydrolytic zinc enzymes also have a role in regulating signalling, e.g. phosphodiesterases that control the degradation of second messengers such as cAMP and cGMP. Lack of zinc in a protein and modulation of function by zinc signalling or redox signalling constitute different mechanisms in the repertoire of zinc regulation.

Signatures of binding sites of inhibitory zinc ions are not readily identifiable by bioinformatics because the ligand donors stem from amino acids located far apart in the amino acid sequence of the protein and are brought into proximity only in the folded protein. Principles of zinc inhibition are that zinc competes for magnesium sites in proteins and that many non-metalloenzymes have a combination of catalytic residues, e.g. Cys, His, Glu,Asp, in the active site, the diads or triads of which provide metal-binding sites. Accordingly, the concept developed that zinc ions not only bind to apoenzymes to form active zinc holoenzymes but also inhibit enzymes that are not zinc enzymes, which then need to be activated by zinc removal [38]. The activation mechanism was suggested to employ thionein, the apoprotein of MT [31]. Proteins do not necessarily have just one type of zinc site. Either two or all three, i.e. catalytic, structural, and regulatory, types of sites can be present. Zinc regulation does not affect only enzymatic functions.

For membrane proteins, the significance of zinc binding and regulations is less clear, because in some cases zinc may bind to sites exposed to the extracellular milieu where zinc buffering is different and has not been characterized. In an extensive list of zinc modulation of ionotropic receptors, most of the activating or inhibiting effects of zinc are in the micromolar range, except for three examples where inhibition has been observed at much lower zinc concentrations: glycinergic (GlyRα1; 80 nM), protonergic (ASIC1a; 7 nM) and glutamatergic (NR1/NR2A; 5 nM) receptors [39]. Here, too, the observations made on isolated systems must be sorted according to their significance for physiology.

The binding of zinc at the interfaces of proteins in protein-protein interactions also depends on zinc concentrations [40]. Whether zinc ion fluctuations are employed to control the assembly of protein complexes is indicated but less well understood. A classic example is insulin, where zinc and calcium ions are needed for the assembly of the insulin hexamer and its crystallization in the dense granules of pancreatic β-cells.

Zinc buffering and hence the pZn value can change in the cytosol as shown for biological processes such as transitions from non-proliferating to proliferating or differentiating cells [19]. The extent to which such changes lead to a re-distribution of zinc in the cytosol and between cytosol and cellular compartments, changes in the expression of zinc proteins, and differences in loading proteins with zinc at their binding sites is also not known.

In summary, zinc, because of its chemical characteristics, has been chosen for the widest possible functions in catalysis, protein structure, and regulation. Its redox-inertness allows it to be used as a signalling ion akin to calcium. It complements calcium biology, but because of its preference for different coordination environments, has different targets and a different action spectrum at lower concentrations. In contrast to the catalytic and structural functions, the significance of regulatory functions of zinc in proteins is not as widely acknowledged. Regulatory functions of zinc will make a significant contribution to understanding cellular biology.

3. The redox activity of zinc-sulfur (thiolate) coordination environments

In contrast to magnesium and calcium, zinc can interact with the sulfur (thiolate) donor ligand of cysteine, which is perhaps the most important factor linking zinc and redox/free radical biology [41]. Zinc thiolate coordination environments can be redox-active [42]. Thus, in contrast to the prevailing dogma that zinc protects thiolates as an “antioxidant”, sulfur can be oxidized/modified in some zinc

thiolate coordination environments. In other words, although the central zinc ion is redox-inert, a ligand-centered redox chemistry can occur and confer redox activity on the complex [43]. It has important consequences for zinc biology as well as redox biology. Zinc binding can be regulated by oxidoreduction, solving the enigma how tightly bound zinc can be released, re-distributed and controlled [44]. The finding challenged the paradigm that zinc proteins are redox-inert and puts zinc proteins with zinc thiolate coordination at the crossroad with redox metabolism. The oxidative release of zinc generates a zinc ion signal that can trigger an antioxidant response. In the simplest case and in analogy to reversible thiol/disulfide equilibria in redox biology, a reversible zinc thiolate/disulfide transition occurs (Figure 5). Depending on the agent and the concentrations of oxidant involved there can be other thiol modifications and oxidation to higher valence states of sulfur, including irreversible oxidation.

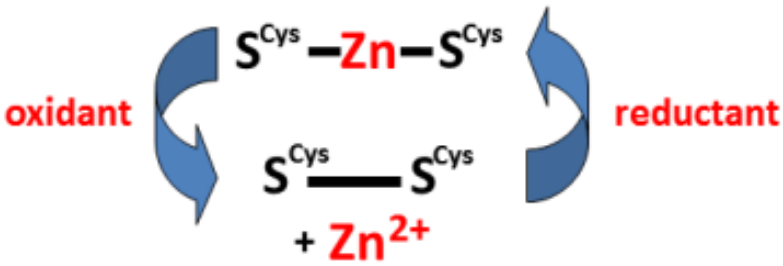

Figure 5 Redox activity of zinc thiolate coordination environments. Zinc dissociates upon oxidation of the cysteine sulfur and increases zinc ion availability while reduction decreases availability. This molecular mechanism (shown here for the formation of a disulfide, which is one of many different products) couples redox and zinc metabolism. Zinc thiolate coordination environments serve as signal transducers to convert redox signals into zinc ion signals for biological control. In this way, they can also serve as redox sensors to allow redox reactions to control catalytic activity and protein structure at sites in or between proteins.

The common thiol/disulfide redox motif CxxC (C = cysteine) is also a metal-binding motif in some zinc finger proteins. It makes redox motifs liable to zinc inhibition. It has been discussed that a conserved *cis*-proline precludes metal binding in some proteins of the thioredoxin family, while in other proteins, e.g. thioredoxin reductases and glutaredoxins, zinc is a potent inhibitor [45,46]. It is a prime example of how specific amino acid signatures have multiple functions: as redox motifs only, zinc-inhibited redox motifs, zinc-binding motifs only, or zinc-binding redox switches.

A theoretical investigation on the reactivity of zinc thiolate complexes with hydrogen peroxide concluded that oxidation depends on the type of ligand and decreases in the order CCCC, CCHC, CCHH (four, three and two cysteines, respectively; H = histidine) due to a lowering of the nucleophilicity of the sulfur ligand donor [47]. Accordingly, the majority of redox-active zinc centers are of the tetrathiolate type [48]. Further discussion of the reactivity of various zinc thiolate sites can be found in a recent review [49].

One protein with twenty-eight zinc thiolate interactions is MT. It will be discussed next because it demonstrates how zinc can express antioxidant functions by providing the cell with additional thiols as reductants. Zinc induces the expression of MT, a 60+ amino acid protein with twenty cysteines. In addition, it is involved in the biosynthesis of glutathione.

3.1 Metallothionein: Prototype of a protein linking cellular zinc and redox metabolism

Metallothionein (MT) binds zinc in two domains in such a way that every zinc ion is surrounded by four cysteine sulfurs. The zinc ions are clustered with three zinc ions bound to nine cysteines in one domain and four zinc ions bound to eleven cysteines in the other. In humans, about a dozen genes encode different MTs. From its discovery in 1957 as a cadmium-containing protein [50], it took at least fifty years to understand its role as a zinc protein in zinc metabolism. The reason for such a long quest for a function is that details of how cellular zinc is controlled were not known until recently and hence the need for a molecule with the zinc-binding characteristics of MT could not have been foreseen. It is now clear that MT buffers zinc exactly over the range of zinc concentrations employed for biological control [51-53]. MT does not have just very tight binding for zinc, which would make it a storage protein, but a range of affinities that cover at least two orders of magnitude [54]. Furthermore, it is not just one protein but a dozen proteins that all underlie specific regulation by many factors including various types of stress, thus adjusting the zinc buffering capacity to the demands of the cell [55].

The reactivity of the cysteine thiolates in MT was shown over 30 years ago. MT reacts with superoxide and hydroxyl radicals [56]. Hydrogen peroxide, oxidants such as hypochlorous acid (HOCl), selenium compounds, and disulfides react with MT and release zinc ions [57-63]. Examination of a host of oxidants showed that the redox potential of the thiols in MT is rather low and that many biological oxidants can release zinc for transfer to other proteins [42, 64, 65]. Nitric oxide also releases zinc from MT *in vitro* and in cells [66-69]. Another class of agents with zinc-releasing potential are electrophiles. Glutathionylated, homocysteinylated, nitrosylated, carbonylated, and drug-modified forms of MT have been described [70-74].

Zinc release from proteins occurs under pathophysiological conditions such as oxidative and carbonyl stress and exposure to drugs and toxicants. It is a way of how reactive agents interfere with zinc metabolism through modification of thiols. The reactivity with zinc thiolate coordination environments in many other proteins has been observed. Therefore, the effect of reactive species on very specific zinc proteins involved in cell biology should not be underestimated. It has been demonstrated that the amount of “free” zinc ions increases with a shift to more oxidizing thiol/disulfide conditions [19]. To which extent this redox biology and the redox biology of reactive species triggers zinc release from MT and from other proteins with zinc thiolate coordination under physiological conditions continues to be an active area of research.

The protein made on the ribosome is thionein (T). The name metallo-thionein merely signifies its association with metal ions. Whether T can acquire zinc to form MT depends on how much zinc is available in the tightly zinc-buffered and zinc-muffled cellular environment. The protein is not always fully saturated with seven zinc ions in cells, suggesting that it should be described in terms of an MT/T ratio [75, 76]. It is also not fully reduced as the oxidized protein (thionin) can be detected in cells [77, 78]. Structural work showed that thionin is in a disulfide form under oxidative stress [79]. The cysteines and their capacity to bind metals ions make the protein both a zinc buffer (MT/T)

and a redox buffer (thionin/thionein) [80]. This property adds a zinc-dependent redox couple to the established zinc-independent redox couples in thiol redox biology. T has the features of an intrinsically disordered protein, which can adopt many different structures upon metal binding or oxidation, and therefore MT has been referred to as a metamorphic protein [54]. The thiols in T have many different orientations and therefore could serve as a sulfur-based chelating agent par excellence to remove zinc from its inhibitory sites in proteins [31]. In addition to the redox control of the chelating capacity at the protein level, gene expression of T is also tightly controlled via multiple signalling pathways, which include redox- and zinc-dependent events and transcription factors, fully integrating the redox and metal biochemistry of MT/T into cellular biology. One lingering question is whether MT is merely a buffer for zinc or interacts with other proteins. The biochemical coupling of MT to at least two other redox systems has been observed. Glutathione is one. Changes in the glutathione/glutathione disulfide couple influence zinc transfer between MT and zinc-requiring enzymes [64,82]. Moreover, redox cycling selenium compounds can catalyse such a transfer *in vitro* [83,84]. The other redox system is methionine sulfoxide reductase, for which T can serve as a reducing agent [85].

When MT is described as an antioxidant, a similar ambivalence exists as for zinc. MT or T may react with oxidants and thus be antioxidants but it is a matter of how much oxidized protein (thionin) exists under various conditions to serve as an oxidant instead and how much zinc is released with either pro-oxidant or pro-antioxidant effects.

3.2 Proteins with zinc redox switches

Redox activity of zinc thiolate coordination environments in many other prokaryotic and eukaryotic proteins has been described and the list of possible candidates amended [43,48]. It introduced zinc into the "redox world." One needs to distinguish, however, whether high oxidant concentrations affect protein functions irreversibly with or without additional adverse effects of the released zinc ions or whether genuine regulation of protein function occurs with either reversible zinc binding regulating the protein and/or zinc being released for a specific purpose.

Disruption of protein function in zinc thiolate coordination environments by sulfur modification has been demonstrated for catalytic and structural zinc sites, e.g. in subunit interactions of dimeric eNOS [86], a voltage-gated K^+ channel (Kv4.1) [87], enzyme inactivation [88] or activation [89], and zinc finger proteins [90]. The consequences are manyfold: catalysis is affected by ejection of zinc or by modification and dissociation of an inhibiting cysteine ligand in the zymogen as in matrix metalloproteinases, protein structure controlled, resulting in changed transcription, protein-protein interaction interrupted, or ion channel function modulated. Sulfur modifications occur under oxidative stress or increased concentrations of reactive species. To which extent such reactions are a way of physiological regulation in higher organisms is not always entirely clear as discussed in themed journal issues [91,92].

Protein kinase C (PKC) is a family of enzymes that are activated by either diacylglycerol or Ca^{2+}, both, or neither one, and regulated by phosphorylation, protein degradation, and redox. Zinc is involved in the function of PKCs in different ways. PKCs have a 2-zinc finger motif (C1 domain) in their regulatory domain. The zinc sites form a zinc redox switch: Oxidants modify the thiolate ligands, release the zinc ions, and activate the enzymes by abolishing the autoinhibitory function of the

regulatory domain [93,94]. Oxidants have a different effect on the catalytic domain, which they inhibit. More recently, it was demonstrated that high concentrations of zinc inhibit PKCδ, a key enzyme in signalling pathways controlling proliferation, differentiation and apoptosis, and interfere with its phosphorylation and translocation [95]. Other key signalling Ser/Thr kinases such as Raf-1 may function with a similar zinc redox switch mechanism [96]. Such a mechanism may also include diacylglycerol kinase, which regulates a switch between two different second messengers and their signalling.

After having treated fundamental aspects of zinc biology with regard to redox biology, the article continues with how zinc is involved in redox metabolism and its regulation, and what happens if the system is perturbed by redox stress, not enough or too much zinc is available, or zinc is added or removed.

4. Zinc in metabolism

The role of zinc in various aspects of metabolism is generally acknowledged [9]. It now seems that zinc enzymes are not simply distributed randomly in metabolic pathways and used wherever a need for zinc in catalysis arises. Instead zinc has more specific roles in the control of metabolism and other aspects of cell biology, not only as a permanent catalytic and structural cofactor of proteins but also in transient interactions with enzymes and proteins that are not zinc metalloproteins. In enzymes, zinc is involved in all six classes defined by the Enzyme Commission. Bioinformatics approaches provided comprehensive lists of human zinc enzymes [15]. In the largest class are hydrolases with 397 members, followed by 302 ligases, 167 transferases, 43 oxidoreductases, and 24 lyases/isomerases. Proteinases include carboxypeptidases, aminopeptidases, matrix metalloproteinases, and peptide hormone processing enzymes/convertases, indicating a wider role of zinc in proteostasis. Hydrolases also include zinc-dependent phosphodiesterases, phospholipases, alkaline and acid phosphatases and pyrophosphatases with roles in regulating second messenger metabolism and signal transduction pathways. Zinc is used in DNA and protein (histone) modification in demethylases and deacetylases, in DNA and RNA metabolism and DNA repair enzymes. Another significant group of zinc enzymes is involved in regulation: transferases such as geranyl and farnesyl transferase, palmitoyl transferases, ligases such as E3 ubiquitin protein ligases, SUMO conjugating enzymes, and the corresponding hydrolases. Zinc is conspicuously absent as a permanent cofactor in the enzymes of the main pathways of aerobic and anaerobic energy metabolism, perhaps, on a speculative note, an indication of an evolutionary pressure not to have these pathways dependent on a micronutrient. However, zinc may be involved in some aspects of regulation of these pathways. For example, the IC_{50} values for inhibition of fructose 1,6-diphosphatase and glyceraldehyde 3-phosphate dehydrogenase are 100 and 150 nM, respectively [31]. Zinc inhibits the aconitase-catalyzed conversion of citrate to *cis*-aconitate with an IC_{50} value of 10 nM, which is important for the accumulation of citrate in prostate epithelial cells [97]. Zinc is part of the committing enzyme of the pathway of porphyrin biosynthesis, δ-aminolevulinic acid dehydratase. Through its catalytic role in carbonic anhydrases it is involved in the acid-base balance/pH control. Some enzymes, e.g. metalloproteinases and alcohol dehydrogenases, have structural zinc sites in addition to their catalytic zinc.

Furthermore, zinc serves as a brace in subunit interactions and in structural sites for organizing protein architecture and interactions. Coupling some of these sites with zinc thiolate coordination

to redox reactions and the dependence of the interactions on zinc concentrations suggests that occupancy of zinc sites is a way of influencing, and maybe regulating, protein conformational landscapes and part of the interactome.

The following selected examples will discuss roles of zinc in some pathways related to redox metabolism. Which proteins are affected when zinc concentrations change or become limiting is difficult to pinpoint as there are so many targets in addition to zinc-dependent regulation of the proteins at the transcriptional, translational and posttranscriptional levels.

4.1. Zinc in coenzyme metabolism

The pterin cofactor pathway leading to tetrahydropterin (BH4), the molybdopterin cofactor, and pteridine in folate/tetrahydrofolate (THF) contains zinc-dependent enzymes. The enzyme initiating the pathway from GTP, cGMP cyclohydrolase I, is a zinc enzyme and the second enzyme, 6-pyruvoyl-tetrahydrobiopterin synthase is also a zinc enzyme. BH4 is needed for the aromatic amino acid hydroxylases in monoamine neurotransmitter biosynthesis and in nitric oxide synthase (NOS).

Pyridoxal kinase and riboflavin kinase, controlling cofactor biosynthesis, prefer a Zn-ATP over a Mg-ATP complex [98]. X-ray structures of these enzymes show the binding of a Zn-ATP complex [99, 100]. The preference for Zn-ATP has also been measured for NAD kinase, which forms $NADP^+$ from NAD^+ [101].

Zinc is involved in other aspects of the metabolism of NAD^+/NADH, which has been called the redox currency of the cell [102]. Mammalian alcohol dehydrogenases are a family of zinc enzymes that use NAD^+/NADH as a cofactor. It includes sorbitol dehydrogenase, which is one of two enzymes in the polyol pathway that performs a transhydrogenation from NADPH to NADH, and, when activated, depletes NADPH and NAD^+.

Sirtuins are directly coupled to the energy status of the cell. They are NAD^+-dependent mono-ADP ribosyltransferases, deacetylases of histones, and have hydrolase activity with a wide array of functions. In addition to having a structural zinc sites, sirtuin1 is strongly zinc-inhibited (IC_{50} = 0.26 nM) [103]. Poly (ADP-ribose) polymerases (PARPs) are signalling enzymes in NAD^+ metabolism. They also use NAD^+ and have a 2-zinc finger motif in their DNA binding domain.

4.2. Zinc in the activated methyl cycle/transsulfuration pathway

In the activated methyl cycle/transsulfuration pathway, zinc has a role in several enzymes (Figure 6) [104]. Betaine-homocysteine methyltransferase and methionine synthase are zinc enzymes. Both enzymes synthesize methionine to provide the cofactor *S*-adenosylmethionine (SAM) for biological methylations. Here again, it is not only the role of zinc in enzymes but also its role in cofactor metabolism and control of enzymes such as cystathionine β-synthase and serine hydroxymethyltransferase by zinc-dependent transcription factors. Adenosine deaminase and guanine deaminase are zinc enzymes forming inosine (hypoxanthine) and xanthine, respectively in the degradation pathway of purine and purine nucleosides leading to uric acid, which is a scavenger for free radicals.

The immediate health implications of the involvement of zinc in the activated methyl cycle are evident with regard to elevated serum homocysteine concentrations as a risk factor for

cardiovascular diseases. Serum homocysteine concentrations are significantly higher in zinc-deficient rats, but zinc supplementation has no effect on this increase [105].

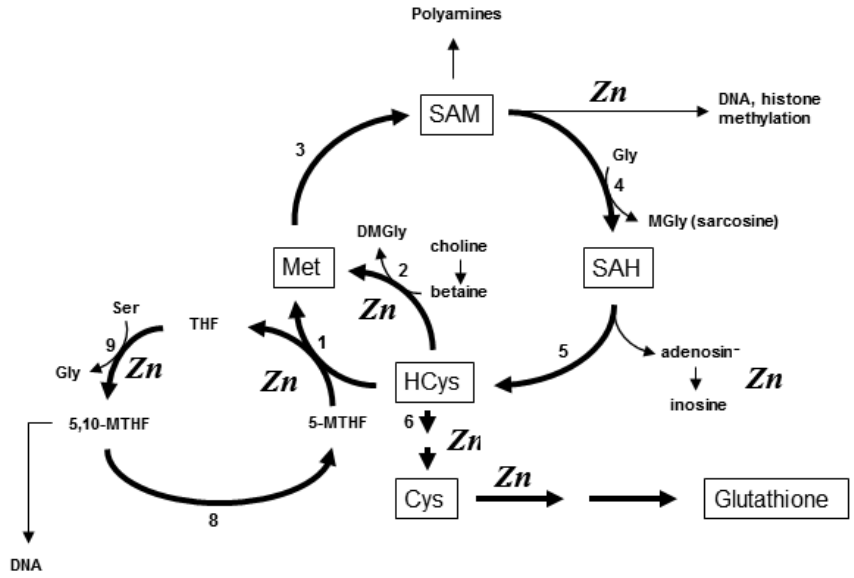

Figure 6 Zinc in the activated methyl cycle/transsulfuration pathway, a critical branching point in metabolism connecting sulfur metabolism, cofactor chemistries, and biological methylations. SAM, *S*-adenosylmethionine, SAH, *S*-adenosylhomosysteine, HCys, homocysteine, Cys, cysteine. *Enzymes: 1*, methionine synthase (MS), zinc enzyme; 2, betaine homocysteine methyltransferase (BHMT), zinc enzyme; 3, methionine adenosyltransferase; 4, N-glycine methyltransferase; 5, SAH hydrolase; 6, cystathionine β-synthetase (CBS), regulated by zinc-dependent transcription factors; 7, γ-glutamylcysteine synthetase (glutamate cysteine ligase, GCL), regulated by zinc-dependent transcription factors; 8, methylenetetrahydrofolate reductase; 9, serine hydroxymethyltransferase (SHMT), regulated by zinc-dependent transcription factors. Additional zinc enzymes are histone and DNA methyltransferases and adenosine deaminase. *Coenzymes:* folate: 5-methyltetrahydrofolate (5-MTHF): reaction 1; 5,10-MTHF: reaction 8; THF: reaction 9; B12: reaction 1; B6: reactions 6 and 9; riboflavin participates in several reactions.

The second aspect of this cycle is that it controls cysteine and glutathione biosynthesis in the transsulfuration pathway. Remarkably, as with MT, zinc and redox regulation converge in glutathione biosynthesis and function. Glutamate cysteine ligase (GCL) is the first, rate-limiting step of glutathione biosynthesis, synthesizing γ-glutamylcysteine from Glu and Cys using ATP. It has two subunits, a catalytic subunit (GCLC), which is active alone, and a modifier subunit (GCLM) which increases the activity of the catalytic subunit. GCLC and GLCM are under control of the transcription factor Nrf-2 (see below). Remarkably, while GCLC is a target of MTF-1-directed gene expression in mice, investigations with primary rat endothelial cells showed that the effect of zinc on GCL synthesis does not depend on MTF-1 [23, 106]. Like MT, glutathione is involved in the interaction of metal ions such as copper and zinc ions and participates in either loading zinc into proteins or zinc transfer between proteins [64, 107, 108].

And a third aspect is that zinc is a cofactor in glutathione-dependent enzymes in the metabolism of reactive species. Alcohol dehydrogenase 5 (class III ADH) reduces formaldehyde in the form of *S*-formylglutathione and is also an *S*-nitrosoglutathione dehydrogenase [109, 110]. Both glyoxalase I, forming *S*-2-hydroxyacylglutathione from 2-oxoaldehdyes and glutathione, e.g. *S*-D-lactoylglutathione from methylglyoxal, and glyoxylase II, which cleaves the thioester, are zinc-containing enzymes in the detoxification of reactive carbonyls.

4.3 Zinc in nitric oxide metabolism

Zinc has a role in reactions that link the urea cycle and NO production. N^{ω}-methyl- (L-NMMA) and N^{ω},N^{ω}-dimethyl-L-arginine (ADMA) inhibit neuronal nitric oxide synthase (nNOS). The enzyme that regulates these inhibitors is dimethylarginine dimethylaminohydrolase (DDAH-1), which forms L-citrulline and mono- or dimethylamine. The enzyme is under the control of the zinc-sensing transcription factor MTF-1 and is inhibited by zinc binding to the active site with a K_i of 4.2 nM [111, 112]. DDAH-2 is the product of another gene and found in tissues expressing eNOS. NOS itself requires zinc for its dimerization, which is interrupted by oxidative modification of the tetrathiolate zinc site (see above). nNOS is inhibited by zinc, but the apparent K_i is only 30 µM [113].

5 Zinc dose-response relationship

Homeostatic mechanisms use sensors that detect imbalances and initiate a programme to restore the steady-state. The sensor for an increase of cellular zinc ion concentrations is MTF-1. The sensor for a decrease of cellular zinc ion concentrations is not known for mammalian systems. In the following section, three ranges will be considered to discuss the effects of zinc on redox metabolism: not enough zinc (zinc deficiency), normal zinc status, and too much zinc (zinc overload). Imbalances can develop as a result of oxidative stress, genetic factors, nutrition, drugs or other agents that bind zinc, or supplementation with too much zinc. Generally, the pro-oxidant effects can be cytotoxic, pro-inflammatory and pro-apoptotic while the pro-antioxidant effects can be cytoprotective, anti-inflammatory, and anti-apoptotic [114].

When quantitative PCR, cDNA array and proteome technologies became available, the effect of zinc on gene expression was examined by increasing or decreasing zinc available to cultured cells. Nutritional zinc deficiency in rodents affected intestinal mRNA levels of MT-1, zinc transporter 2, uroguanylin, and iNOS as well as mRNAs of genes involved in signalling pathways, growth, transcription, redox and energy utilization [115, 116]. In the human colon cancer cell line HT-29, lower intracellular zinc caused changes in the mRNA levels of 309 genes [117]. The transcripts encode proteins in intermediary metabolism (79) including protein metabolism (21), signalling (30), cell cycle control and growth (15), vesicular trafficking (15), cell-cell interactions (13), cytoskeleton (10) and transcriptional control (19). Higher intracellular zinc impaired ATP synthesis and cell cycle control and induced proteins involved in the stress response [118]. Another global analysis of the effects of zinc deprivation and excess on human monocytic/macrophage THP-1 cells demonstrated that 5% of 1,045 genes investigated were zinc-responsive [119]. In addition to zinc-dependent gene transcription, there are numerous effects on cellular functions that do not depend on de novo synthesis of proteins.

5.1 Zinc deficiency, a pro-oxidant condition causing oxidative stress

The effect of zinc deficiency on growth of *Aspergillus niger* was reported in 1869 by Jules Raulin, a student of Louis Pasteur [120]. Evidence for zinc deficiency in humans was gathered only in 1963 [8]. Over these hundred years, it became established that zinc is needed for the growth and development of all species. The involvement of zinc in a myriad of proteins provides a basis of why zinc deficiency affects so many physiological functions.

A hallmark of zinc deficiency is oxidative stress. The primary cause for the oxidative stress and the chain of biochemical events in zinc deficiency is not entirely clear. General features, however, have been established. Overall, consequences of zinc deficiency are an inability to launch an antioxidant defence, which at least in part depends on zinc as shown for hydrogen peroxide [121], generation of endoplasmic reticulum (ER) stress, an inflammatory response, and an effect on cellular calcium.

In zinc-deficient rodents, the function of calcium channels in the plasma membrane is compromised, perhaps due to changes of the redox state of critical thiols, leading to a reduction in calcium uptake [122]. Depleting extracellular zinc results in a rapid transient mobilization of intracellular "free" zinc ions [123]. A change of the thiol/disulfide redox state to more oxidizing conditions also increases cellular "free" zinc ion concentrations [77]. Zinc inhibits the plasma membrane Ca^{2+}-ATPase, which controls cellular free calcium concentrations, with a K_i of 80 pM [124]. Indeed, zinc deficiency in animals depresses Ca^{2+}-ATPase activity in erythrocyte membranes [125]. Also, an increase of cellular "free" zinc ions to concentration >2 nM, as observed in ischemia, leads to calcium leakage from the cardiac redox-sensitive ryanodine receptor/calcium channel (RyR2) [126]. The increase of cellular calcium activates NADPH oxidase and NOS and results in the production of reactive species. Extracellular zinc deprivation also activates the production or reactive species by NADPH oxidase and NOS [127, 128]. Zinc, together with copper, is a cofactor of the active site of superoxide dismutases 1 and 3. Under zinc deficiency, SOD undergoes a conformational change that makes the interaction with the protein Derlin possible and activates the ER stress response [129]. Taken together, these results delineate a plausible pathway how less available extracellular zinc affects intracellular interactions between zinc and calcium metabolism and causes oxidative stress.

Zinc protects isolated lysosomal and microsomal preparations from lipid peroxidation [130, 131]. Subsequently, it was shown that zinc deficiency leads to membrane damage in isolated organelles [132], protein and DNA damage in isolated cells in addition to its effects on lipid peroxidation [133, 134], and changes in signal transduction pathways that determine both cell fate and protection of cells against stress [135]. All of these effects are due to zinc deficiency eliciting an oxidative stress in cultured cells [136]. And finally, it was confirmed that markers of oxidative stress increase under suboptimal zinc intake in humans [137-139]. Because of its DNA damaging effects and the role of zinc in DNA repair, zinc deficiency was discussed as having a role in carcinogenesis [140]. Work on esophageal squamous cell carcinoma demonstrated that zinc deficiency is a risk factor. Zinc deficiency causes an inflammation with a dose-dependent up-regulation of oncogenic mi-RNA [141].

When reviewing the oxidative stress in zinc deficiency, the role of the transcription factor Zap1 as a sensor of low zinc status in yeast was discussed [142]. Among the about 80 genes induced is TSA1, a peroxiredoxin that reduces hydrogen peroxide to water through reactions coupled to thioredoxin, thioredoxin reductase and NADPH. Zap1 is also involved in controlling genes that are needed for sulfur assimilation in yeast with an additional posttranslational control of these proteins that are involved in providing methionine and cysteine in an NADPH-dependent pathway. Under zinc-

limiting conditions, the levels of these sulfur amino acids decrease. This situation is reminiscent of the role of zinc in the antioxidant response and the transsulfuration pathway in humans (see above). However, a transcription factor such as Zap1 in yeast is not known to exist in humans. Oxidative stress sensing seems to be involved in detecting low zinc concentrations with a transcriptional response controlled by MTF-1 and Nrf-2 (see below).

Further investigations with the yeast model began to address the effects of zinc deficiency on the zinc proteome. They showed that transcriptional regulation results in a lower abundance of zinc proteins and that many zinc enzymes are made but not metalated or perhaps mis-metalated [143]. It remains unknown whether kinetic or thermodynamic factors determine the re-distribution of zinc and whether a functional hierarchy exists and is managed by a triage process that prioritizes the allocation of zinc. A triage hypothesis has been proposed for the systemic control of micronutrients. It distinguishes allocation to proteins necessary for immediate survival and reproduction from that to proteins important for long-term health [144].

In summary, minimally there are two pathways for generating oxidative stress in zinc deficiency, a direct pathway mediated through an effect on calcium metabolism and an indirect pathway limiting the antioxidant response. One would assume that the regulatory functions of zinc cease first under zinc deficiency. It is supported by the observation that cells stop growing and differentiating. If zinc is further restricted, proteins will not obtain their structural and catalytic zinc for function. In addition, oxidative stress will interfere with their functions. When added under pro-oxidant condition of zinc deficiency zinc will have antioxidant-like functions. However, whether homeostatic control of zinc can be completely restored will depend on whether the oxidative stress, which can originate from zinc-independent pathways, can be controlled and redox homeostasis be restored.

A consequence of low zinc and the associated oxidative stress is systemic chronic inflammation and eventually cell death. One of the first signs of zinc deficiency is a compromised immunity. Both innate and adaptive immunity depend on zinc in many different ways [145].

5.1.1. Zinc and inflammation

Inflammation is a response of the host to pathogens or injury. A component of it is the acute phase response as a result of stress or infection. It lowers the zinc concentration in the blood. Acute and chronic inflammation is associated with additional changes in zinc metabolism. Activation of Toll-like receptors on immune cells or the tumor necrosis factor α (TNF α) receptor on other cell types results in signal transduction to the transcription factor NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells), which controls the production of cytokines and the survival of cells. Signalling to NF-κB is zinc-dependent [146]. An examination of the NF-κB signalling pathway revealed the involvement of zinc in at least 25 sites of different proteins (TRAF2, A20, Tab2/3, TAX1BP1, Nup475/TTP/Tis11, IKKγ/Nemo, RNF11 [114]. This observation epitomizes the prevalence of zinc in signalling pathways and hence the issue how difficult it is to pin-point which site(s) will be affected by zinc deficiency primarily or which proteins zinc regulates in this or any other signalling pathway. Zinc deficiency has a pro-inflammatory effect while optimal zinc concentrations suppress the inflammatory response. In humans, inflammatory cytokines increase in zinc deficiency. Thus, in addition to zinc being a constituent of the proteins in the NF-κB signalling pathway, it can be involved in the regulation of proteins at various levels. A transcriptional target of NF-κB is the zinc

transporter Zip8 which transports zinc into the cell and modulates IκB kinase reversibly to trigger an anti-inflammatory response [147].

These findings are also highly significant for endothelial dysfunction and the involvement of zinc in the inflammatory process and ensuing vascular disease [148]. Zinc deficiency in vascular endothelial cells increases the binding of NF-κB to DNA, induces expression of cyclooxygenase-2 and E-selectin and affects monocyte adhesion [149]. Transactivation of peroxisome proliferator-activated receptor (PPARγ), which inhibits NF-κB/DNA binding, requires zinc. Therefore, under zinc deficiency there is no inhibitory action and inflammatory cytokines are produced [150]. PPARs belong to the nuclear hormone receptor family which have two tetrathiolate zinc fingers, which themselves are sensitive to oxidative stress. These interactions also demonstrate that zinc status can be critically important for drug action. Rosiglitazone is an agonist for PPARγ. Under zinc deficiency the drug is expected not to work properly [149].

5.1.2. Zinc and cell death

Zinc is involved in cell fate decisions [151]. Zinc is needed for cell growth, and without enough zinc, there is cell cycle arrest at G1/S and G2/M checkpoints. From both checkpoints zinc deficiency can cause apoptosis, which normally is suppressed by a balanced zinc status [152]. There is significant insight into how zinc deficiency causes apoptosis. Forty years ago, it was noted that zinc deficiency in rodents leads to apoptosis in the small intestine and in the thymus, resulting in an immunodeficiency [153, 154]. Zinc deficiency in rodents accelerates apoptosis among pre-T cells and pre-B-cells [155]. In zinc-deficient mice, a chronic elevation of glucocorticoids (corticosterone), which can be prevented by adrenalectomy, was observed and it was demonstrated that zinc deficiency is a condition of stress [156,157]. Glucocorticoid receptors also belong to the nuclear hormone receptors with two tetrathiolate zinc fingers sensitive to oxidative stress. In epithelial cells of fish gill cells, cortisol increases the uptake of zinc, the increase of “free” zinc ion concentrations and the MTF-1-dependent expression of ZnT-1 and two MTs [158]. Glucocorticoids also affect cognitive functions. It has been discussed how the increased levels of glucocorticoids under zinc deficiency alter brain functions associated with zinc signalling [159].

Caspase-3 is activated in zinc-deficient cells and in rat embryos [160, 161]. Zinc was found to inhibit caspase 3 with an IC_{50} value of 0.15 μM [162], but later the IC_{50} was determined to be as low as 10 nM [31], suggesting that the inhibitory zinc must be removed to initiate apoptosis.

A remarkable structural basis with complex regulation of caspases has now been established, indeed confirming zinc binding and the need for activation by removal of the inhibitory zinc in some caspases [163]. Caspases-3, -6, and -8 are inhibited at cellular zinc ion concentrations (2.6-6.9 nM) whereas inhibition of caspase-7 is weaker (76 nM). Capase-3, -7 and -8 bind three, one and two zinc ions, respectively, at the active site, while zinc inhibits caspase-6 allosterically. Caspase-8 is inhibited at the active site but also at a second site, inhibiting the formation of the active dimer. The example of caspases shows how zinc inhibition employs different strategies and affinities for regulation.

Structural zinc sites are also found in inhibitor of apoptosis proteins (IAPs) and BAK (BCL-2-antagonist/killer 1), a pre-apoptotic protein from the BCL-2 (B-cell lymphoma 2) family of proteins that are needed to permeabilize the outer mitochondrial membrane to release apoptotic factors in the intrinsic pathway of apoptosis. For instance, XIAP (IAP3/BIRC3) has a BIR (Baculovirus Inhibitor

of apoptosis protein Repeat) domain with a 3Cys/1His binding site and controls caspases -3, -7, and -9. It also has a RING zinc finger domain involved in ubiquitination of caspases. The BAK homodimer binds a zinc ion at its dimer interface [164].

The tumour suppressor p53 is a cellular guardian that detects DNA damage and potentially damaging stress, including oxidative stress, and decides between a DNA repair programme and cell cycle arrest and apoptosis. It is a transcription factor with a zinc thiolate coordination site in the transactivating domain. It is controlled by interaction with MDM2 (mouse double minute 2 homologue), which is a U3 ubiquitin ligase with a RING zinc finger and blocks the transactivating domain of p53.

A major review article discusses the molecular events pertinent to redox signalling under zinc deficiency as investigated in rodents and in cultured cells [165]. The effects mirror many of the observations made when the zinc levels are too high, indicating the same signalling pathways and the role of zinc in controlling the phosphorylation of proteins. But there are also differences. One important observation in this regard is a deficit in microtubule assembly under zinc deficiency and consequently disrupted cytoplasm-nucleus shuttling of transcription factors [166].

In rats with maternal marginal zinc deficiency and in the IMR-32 neuroblastoma cell line grown under zinc-limited conditions, JAK(Janus kinase)/STAT (signal transducer and activator of transcription) signalling is affected, in particular a reduced tyrosine phosphorylation of STAT1 and STAT3 and hence less transactivation. α-Lipoic acid prevents the reduced phosphorylation of STAT1 but not STAT3, perhaps indicating differential effects of redox and zinc modulation of protein tyrosine phosphatases [167].

There is a considerable literature linking zinc and caspase-independent cell death involving lysosomes and autophagy [168]. In addition to the many zinc transporters coordinating subcellular zinc traffic, other membrane transporters also are involved in translocating zinc ions. There is oxidative release of zinc from lysosomes through TRPM2 (transient receptor potential cation channel subfamily M, member 2) leading to pancreatic β-cell death [169]. TRPM7 is located on a novel type of vesicle, M7V. It is distinct from endosomes, lysosomes or other vesicles, stores only zinc and glutathione, and releases its contents as a function of oxidation [170]. Unrelated to these findings on cellular organelles, another channel from this family, TRPA1 (subfamily A, member 1), is activated by zinc ions with an EC_{50} value of 7.5 nM and serves as an intracellular sensor for zinc in dorsal root ganglia neurons [171]. Similar findings were reported for the same somatosensory receptor in other tissues, requiring zinc influx through the channel and activation by zinc intracellularly [172].

The state of zinc in lysosomes and zinc-mediated autophagy is important for both zinc deficiency and zinc overload (see below). Thus, it is a matter of whether an oxidative stress comes first and triggers zinc release or a zinc stress comes first and triggers an oxidative stress. Under conditions of oxidative stress, zinc concentrations increase in lysosomes, presumably as a result of zinc being released from proteins such as MTs, inducing lysosomal dysfunction by membrane permeabilization and autophagy [173]. One major pathway of zinc-induced apoptosis is the activation of PARPs, a process which uses relatively large amounts of NAD^+, which then leads to a decrease in ATP production.

Another compartment that is discussed regarding zinc deficiency and zinc overload is the ER where not enough or too much zinc cause protein misfolding and the unfolded protein response (UPR), signalling ER stress.

5.2 Zinc as a pro-antioxidant

Two different conditions can be distinguished. First, the physiological zinc concentrations already present counteracting pro-oxidant conditions by inducing an antioxidant response, in which case zinc acts as a pro-antioxidant. And second, the effect of supplemented zinc or zinc added to cultured cells, which can protect against an insult/stressor. There is no evidence that zinc ion signals are used to generate a pro-oxidant redox signal under physiological conditions.

An "antioxidant paradox" was noted on the ground that dietary zinc does not have the same actions as cellular zinc [174]. The argument is based on the fact that extra zinc in the diet (3-10 x recommended daily allowance, RDA of 15 mg for males 11-50 years of age) competes with copper uptake, reducing the activity of erythrocyte Cu,Zn-SOD in humans and liver and heart Cu,Zn-SOD in animals and thus has a pro-oxidant effect. On the other hand, an antioxidant role is believed to be due to an increase of the zinc-MT pool. Another article entitled "Why zinc should not be considered an antioxidant" points out that zinc is also a selenium antagonist, abolishing its anticarcinogenic effects and that additional zinc in the diet may be linked to an increase in skin cancer and that additional zinc in healthy human breast tissue increased the risk of later breast cancer development [175]. A similar observation was made for prostate cancer where the risk increased slightly when 2.5 times the recommended tolerable upper limit of 40 mg was supplemented in trials to prevent age-related macular degeneration [176].

Three mechanisms have been adduced to explain the pro-antioxidant functions of zinc: a) protection of free sulfhydryls, b) out-competing redox-active metal ions, and c) specific induction of an antioxidant response. The first two do not seem to explain antioxidant-like function because zinc thiolates are redox active and zinc binds to the active site of antioxidant thiol enzymes and inhibits them. Also, too much zinc can induce a copper deficiency, which results in a pro-oxidant response due to the need for copper in SOD. But there are also processes that, with or without supplementing zinc, make more "free" zinc ions available in the cell, e.g. processes that affect cellular zinc homeostasis and metal buffering, engendering a pro-antioxidant effect. For example, "free" zinc ion concentrations transiently increase in preconditioning of cortical neurons. The excitotoxic tolerance associated with this preconditioning involves PKC-induced phosphorylation of MT and modulation of gene expression [177]. Thus, for the third mechanism there is clear evidence in two pathways.

MTF1. The transcription factor MTF-1 binds to an MRE (metal response element) in a zinc-dependent way. It has six zinc fingers which are thought to participate in the zinc-sensing mechanism in addition to a metal-responsive activation domain. MTF-1 is part of a stress response to heavy metals, hypoxia and oxidative stress, maintenance of cellular zinc homeostasis by induction of MT and ZnT1, and it is essential for embryonic liver development [23]. Various stressors such as cadmium or oxidants react with MT and release zinc, which then activates MTF-1. In this way, MT has an indirect effect on metal metabolism as an "antioxidant." Other important genes induced by zinc activation of MTF-1 are involved directly in redox metabolism: selenoprotein W (basal expression), a protein involved in growth, differentiation, and protection from oxidative stress;

selenoprotein H (repressed), a nuclear oxidoreductase with functions in carcinogenesis and mitochondria, and in suppressing senescence; thioredoxin reductase-2 (repressed), a mitochondrial form of the enzyme, scavenging reactive oxygen; GCLC (heavy chain, basal expression); placental growth factor, which is hypoxia-induced; dimethylarginine dimethylaminohydrolase, involved in NO metabolism as stated above; α-fetoprotein, a fetal analogue of serum albumin, which is a zinc binding protein (both basal expression); enhancer protein C/EBPα, a protein involved in liver development; C-reactive protein (CRP) which has LIM zinc-binding domains; and KLF4, a zinc finger protein involved in many fundamental processes.

Nrf-2 (nuclear factor erythroid 2-related factor 2). The transcription factor Nrf-2 binds to an ARE (antioxidant response element). Among the genes induced are NAD(P)H: quinone oxidoreductase 1, heme oxygenase 1, and enzymes in the antioxidant defence such as glutathione peroxidase 2, peroxiredoxin 1, thioredoxin and thioredoxin reductase, and GCL. Nrf-2 interacts with Keap1 (Kelch-like ECH associated protein-1), which keeps Nrf-2 inactive and ready for proteosomal degradation. Keap1 is a sensor for electrophiles. The sensing leads to dissociation and activation of Nrf-2 transcriptional activity stimulated by PKC. It has been postulated that a zinc tetrathiolate site is involved in sensing electrophiles [178]. It was also suggested that Nrf2 is a sensor for zinc when employing residues His-225, Cys-226 and Cys-613 for zinc binding. In order to demonstrate this zinc sensor function, which involves a conformational switch associated with zinc binding, cultured cells were exposed to relatively high, micromolar concentrations of zinc ions [179, 180].

Many investigations have shown that supplemental zinc protects against stress injury in organs such as the liver and the heart. In the elderly, zinc supplementation improves markers of oxidative stress and inflammation [139]. The main mechanism underlying these observations seems to be the antioxidant defence induced via these two transcription factors.

In summary, zinc is involved in controlling reactive species and the antioxidant response under physiological concentrations and this function can be enhanced by additional zinc under some conditions.

5.3 Zinc overload: A pro-oxidant condition

If the discussion was challenging which processes, governed by an estimated 3000 zinc proteins, are affected in zinc deficiency, a corresponding discussion what happens when cellular zinc concentrations increase is even more daunting. As zinc ion concentrations increase more interactions become possible. The consequences depend on the zinc buffering and muffling capacities of cells, which likely differ in cells depending on the variable expression of MTs and zinc transporters. Zinc interacts strongly with proteins and, if not properly controlled, the lack or a surplus of zinc can cause protein misfolding and aggregation.

In stark contrast to zinc not being very toxic systemically, it is relatively toxic for cells when unbuffered and released intracellularly. For investigations with cultured cells, it is important to consider that normal zinc concentrations in blood are about 15 µM and buffered and even lower in the extracellular fluid (about 5 µM). Serum-free media have very little buffering capacity and except for F-12 medium do not contain added zinc. Accordingly, toxicity to cells can be detected at concentrations as low as 20 µM added zinc, whereas zinc concentration > 100 µM become toxic in media with 5-10% serum [181]. If agents are present that serve as ionophores, toxicity is expressed

at much lower concentrations. Thus, thymocytes cultured in the presence of 10% serum undergo apoptosis when exposed to only a few hundred nanomolar zinc pyrithione [182]. The scientific literature is replete with experiments performed at much higher, occasionally even millimolar, concentrations of zinc. HeLa cells can adapt to high zinc concentrations (200 μM) and become resistant to zinc [183]. They express more MT and consequently become more resistant to oxidative stress. Other differentially expressed proteins in these adapted cells include cochaperones, oxidoreductases, and ubiquitin [184].

The term "zinc overload" is not well established. It can refer to i) zinc supplemented at high doses (>100 mg/day), notwithstanding that supplementation at much lower doses in a healthy individual can have adverse effects by reducing copper and iron uptake, ii) increases of cellular "free" zinc ion concentrations if the buffering and muffling capacities are exhausted or challenged by other means such as exposure to toxic metal ions, and iii) genetically determined conditions. For example, cases of unusually high serum zinc concentrations have been reported. One condition is due to calprotectinemia, elevation of a protein of the S100 family, the expression of which is controlled by zinc in myeloid cell lines [185, 186]. Investigations in two areas influenced our knowledge about zinc as a cellular toxin: exposure of the lung to inhaled zinc-polluted air and intrinsic dislocation of zinc ions damaging neurons in the brain.

Occupational and environmental exposure to zinc-containing particles through inhalation can result in supraphysiological zinc ion levels that activate gene expression to mount a defence and an inflammatory response and that cause oxidant conditions through the production of reactive species and concomitant changes of the glutathione/glutathione disulfide ratio. Mitochondrial dysfunction and modulation of the mitochondrial permeability transition pore eventually lead to cell death signalling [187]. In substantial contributions from the authors in publications summarized in their review, the effects of zinc were mapped to several pathways. Zinc activates mitogen-activated protein kinase (MAPK)-dependent pathways: JNK (c-Jun-terminal kinase)/p38, and ERK (extracellular signalling kinase). Zinc affects the epidermal growth factor receptor (EGFR) pathway that also activates NADPH oxidase. Interleukin-8 (IL-8), a neutrophil chemotactic factor, cyclooxygenase-2, and heme oxygenase (HO-1) increase through activation of Keap-1/NF-κB dependent transcription in human airway epithelial cells. Central to these events is an effect of zinc on phosphorylation signalling. In addition to effects of zinc on kinases, zinc inhibition of protein tyrosine phosphatase 1B was demonstrated [188, 189].

Work on how zinc affects the developing central nervous system and what roles it has in the injured and inflamed brain and in neurodegeneration is even more prolific and covered by many extensive reviews [190-192]. Initially, calcium overload was shown to be the link between glutamate excitotoxicity and neurodegeneration in ischemia. But then zinc was discussed as the downstream mediator of the damage with mitochondrial dysfunction as having a major role [193]. Some neurons have zinc-rich vesicles (boutons) in their synaptic terminals and use zinc for neurotransmission. Regional differences in the sources of zinc ions and their interactions with mitochondria in neurons exist and underlie differential vulnerability in diseases [194]. Activated microglia are a source of reactive species, which can increase "free" zinc ions in neurons. As a consequence of the increases in cellular zinc ions, potassium efflux occcurs and leads to neuronal death [195]. A decline in ATP production due to a decrease in NAD^+ via zinc inhibition of glyceraldehyde phosphate dehydrogenase and PARP activation contributes to cell death [196]. In essence, an increases of

cellular “free” zinc ions downstream of increased cellular calcium is a major contributor to neuronal death (Figure 7). Activation of NADPH oxidase, NOS, and release of zinc ions from proteins such as MT, and perhaps from organelles, and zinc inhibition of the mitochondrial respiratory chain and ensuing generation of reactive species are the major factors in causing cell death [197, 198]. Pathways involving phospholipase A2 and lipoxygenase or xanthine oxidase have been discussed as a source of reactive species in some cells.

Lysosomes, the ER and mitochondria contribute to cell death when zinc homeostasis is perturbed. Under conditions of oxidative stress, zinc concentrations increase in lysosomes, presumably as a result of zinc released from proteins such as MT and induce lysosomal dysfunction by membrane permeabilization and autophagy [199].

Last but not least, the role of zinc in mitochondrial function will be addressed briefly. The role of mitochondria in energy metabolism, production of reactive species, and apoptosis is highly significant for conditions of zinc overload. What happens to mitochondrial function in zinc deficiency and whether zinc modulates mitochondrial function under normal physiological conditions needs further exploration. Zinc is a constituent of some of the protein complexes of the respiratory chain. It also inhibits mitochondrial respiration by interacting with sites in complexes I and III [200, 201]. Complex I has a zinc site but the binding is rather weak with a dissociation constant of about 10-50 µM [202]. Zinc also interacts with His-503 in bovine cytochrome c oxidase (complex IV) and inhibits proton pumping [203]. MT carries zinc through the mitochondrial outer membrane and releases zinc in the intermembrane space. The released zinc then inhibits the respiratory chain, decreases ATP synthesis and increases the production of reactive oxygen, affecting the mitochondrial permeability transition pore and releasing apoptotic factors [204-206]. Zinc inhibition of mitochondrial respiration (α-ketoglutarate supported) affects the lipoamide dehydrogenase reaction of the α-ketoglutarate dehydrogenase complex with a K_i of about 150 nM, activating the NADH oxidase reaction and leading to hydrogen peroxide and superoxide formation with an activation constant of about 90 nM [207].

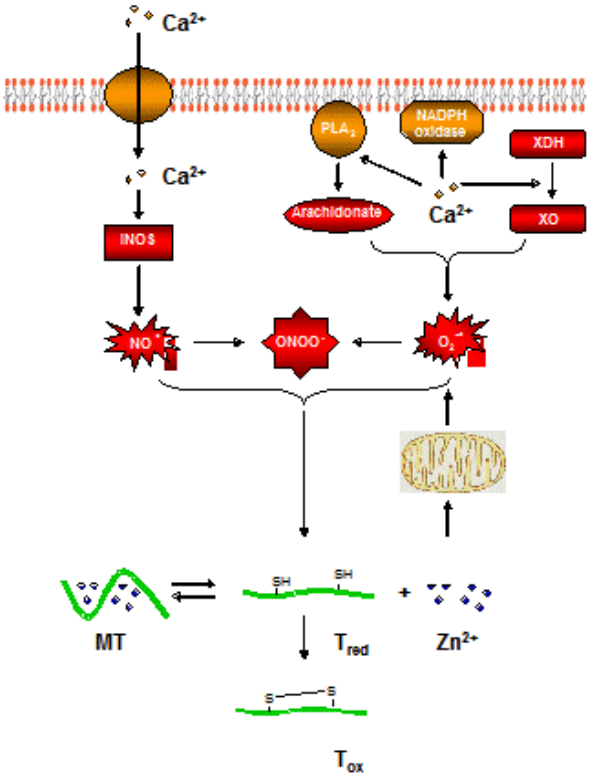

Figure 7 The role of zinc in neurotoxicity. Calcium entry into neurons activates iNOS for the production of nitric oxide (NO) and at least three additional pathways, phospholipase A2 (PLA_2), NADPH oxidase, and xanthine oxidase (XO) for the production of superoxide (O_2^-). The NO and O_2^- radicals combine to form peroxynitrite ($ONOO^-$). The reactive species affect metallothionein (MT), thiol/disulfide buffering of thionein (Tred) and thionin (Tox) and other thiol/disulfides such as glutathione/glutathione disulfide and increase the concentrations of "free" zinc ions that inhibit mitochondrial respiration, decrease ATP synthesis, and increase the production of reactive species.

6 Implications for the life course. Diseases and healthy ageing

The relationship between redox and zinc biology has enormous implications for understanding cellular biology in health and disease. As discussed in this article, zinc deficiency causes oxidative stress and oxidative stress inevitably affects cellular zinc metabolism. Oxidative stress can lead to a loss of zinc and possibly zinc deficiency, which then exacerbates oxidative stress and initiates a vicious circle. Thus, all conditions associated with oxidative stress, i.e. atherosclerosis and cardiovascular disease, obesity and diabetes, ischemia-reperfusion, cystic fibrosis and other fibrotic processes, rheumatoid arthritis and inflammation of other organs such as the liver, brain and lung (chronic obstructive pulmonary disease, COPD), cancer, and brain conditions such as ischemia/stroke, traumatic injury, neurodegenerative diseases (Parkinson and Alzheimer disease), amyotrophic lateral sclerosis, microbial and viral infections [3], need to be considered with regard to the consequences for zinc metabolism and a role of zinc in pathogenesis. In addition, conditioned zinc deficiency occurs as the result of many diseases, i.e. malabsorption syndrome, liver disease, chronic renal disease, sickle cell disease, and other chronic illnesses [208]. The extensive literature with excellent reviews on oxidative stress in most of these diseases should prompt the reader to "think zinc" in all cases. The physician should evaluate the zinc status and consider proper zinc

nutriture and supplementation as therapeutic approaches or preventive measures as zinc has been shown to be efficacious in protecting several organs and tissues against stressors, inflammation and fibrotic processes.

The relationship is also important for toxicology and pharmacology with many examples of environmental pollutants, toxins, or drugs interfering directly with zinc metabolism, conditioning a zinc deficiency or inducing oxidative stress. Nutrition has a primary role in determining the antioxidant status in the first place. In as much as there are genetic factors that affect redox biology, numerous mutations in the proteins controlling zinc homeostasis have been discovered recently [208]. It will be exciting to learn how these mutations affect redox metabolism.

In addition to many of the listed diseases increasing with age and being considered age-related, there is the overarching issue that ageing itself has always been linked with oxidative stress. Very pertinent to etiology and ageing is that zinc deficiency develops in the elderly with a decline of the immune system and a low grade inflammation as documented in some but not all countries [210]. Over the entire life course, zinc deficiency is wide-spread and a significant factor for morbidity and mortality in the developing and even the developed world. It has been estimated that 82% of pregnant women are zinc-deficient, which can be a serious risk factor for child development and physical and mental health later in life [211].

A major difference to iron metabolism, for which many clinical markers exist and imbalances are clinically much more readily defined, is that the only general clinical marker for zinc status is serum zinc. It does not necessarily reflect zinc status in tissues and cells because zinc in blood is only 0.1% of the total. Finally, there is the issue that zinc supplementation, while indicated when plasma zinc is low, has met with mixed results in many of the diseases accompanied by oxidative stress. Based on the foregoing discussions, oxidative stress may need to be controlled in order to restore zinc-dependent functions efficiently through zinc supplementation [212].

7 Conclusions

A molecular basis is emerging for significant roles of signalling zinc ions in cellular regulation and redox homeostasis (Figure 8). Redox and zinc biology are linked and tightly controlled to perform fundamental biochemical functions. The cellular redox and zinc states are dynamic. Induced fluctuations are used for signalling. The article attempted to show how important zinc is for redox biology, namely that zinc deficiency causes oxidative stress, oxidative stress causes zinc re-distribution, and the redox activity of zinc thiolate coordination environments impacts protein structure and function. Specifically, redox modulation confers coordination dynamics on zinc finger sites that are perceived as permanent fixtures of proteins [213]. The relationship between zinc and redox biology has enormous implications for physiology and virtually all diseases associated with oxidative stress. From the original observations of damage to biomolecules and generation of reactive species under zinc deficiency the notion developed that zinc has antioxidant-like properties. The postulated mechanisms underlying such an action, namely that zinc protects sulfhydryls from oxidative modification and that zinc suppresses the generation of free radicals by outcompeting redox-active transition metal ions, is contestable. Zinc-bound thiolates are not necessarily protected but reactive. Zinc has a role in regulating the antioxidant defence by restoring the redox poise and controlling the synthesis of reducing thiol-containing biomolecules such as metallothionein and glutathione via activation of MTF-1-dependent gene transcription. Competition with iron and

copper occurs outside cells during uptake of metal ions but inside cells metal ions are tightly buffered and it is less the competition between metal ions but rather the interdependence of their metabolism that is important for redox biology. The biological significance of zinc is evident when compared to iron: While 2% of all proteins utilize iron as a cofactor, 10% of all proteins utilize zinc in structure and catalysis. The regulatory functions of signalling zinc ions on proteins are in addition to the functions of zinc in a myriad of zinc proteins and biomolecular interactions and to the cellular control performed by calcium signalling. To distinguish physiological from pathophysiological fluctuations of zinc ion concentrations it is necessary to measure the affinity of proteins for zinc and the very low concentrations of signalling Zn^{2+}, which are three orders of magnitude lower than those of Ca^{2+}. Such determinations became possible only with designed fluorescent probes and sensors and continue to be challenging [214]. Advances will come from further mapping the chain of events in signalling and evaluating the different concentration ranges of zinc ions and their targets as physiologically, pharmacologically or toxicologically significant. The majority of investigations have been performed with isolated proteins and cultured cells. Translation to the situation in humans for improving health is lagging behind and will require specific marker for cellular zinc status and new types of interventions. One would hope that closer interactions between the communities of zinc biologists and redox biologists will foster the adoption of methods and protocols to measure simultaneously redox and zinc species with the aim of understanding their joint contributions to health and disease.

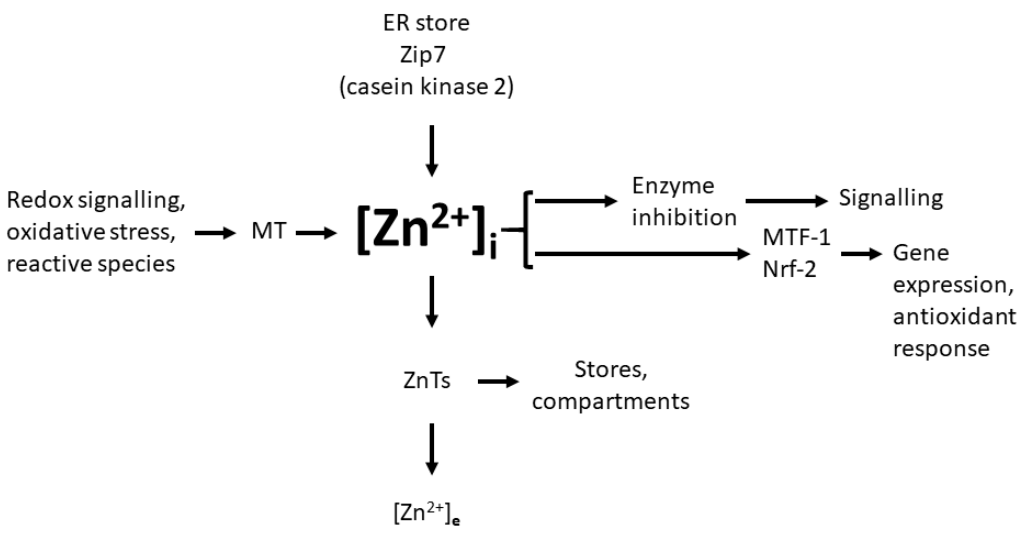

Figure 8 Zn^{2+} in cellular regulation and interdependence with redox signalling and redox metabolism. Modification of thiols in metallothionein (MT) and phosphorylation of the zinc channel Zip7, a gatekeeper of a zinc store in the ER, increase cellular "free" zinc $[Zn^{2+}]_i$, generating a zinc signal that affects key enzymes in signal transduction pathways and activates MTF-1- and Nfr-2-dependent gene transcription that includes an antioxidant response involving thionein (T) and enzymes of glutathione synthesis. Zinc transporters (ZnTs) dampen the zinc signal, store zinc in organelles, specific exocytotic vesicles, or export any surplus. Vesicular exocytosis of zinc generates extracellular zinc signals $[Zn^{2+}]_e$ for various purposes. The system that controls zinc signals is part of the overall system of cellular homeostatic control.

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Footnote (page 3)

† The term oxidative stress for a redox imbalance is widely accepted. It comprises different situations: reductive stress, a surplus of reactive species, and effects on redox signalling.

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